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(54) Recombinant secA2 polypeptide from Staphylococcus aureus and uses thereof

- (57) The invention provides secA2 polypeptides and DNA (RNA) encoding secA2 polypeptides and methods for producing such polypeptides by recombinant techniques. Also provided are methods for utilizing secA2 polypeptides to screen for antibacterial compounds.

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Description

RELATED APPLICATIONS

- 5 This application claims benefit of Great Britain patent application number 9604045.6, filed February 26, 1996, and U.S. Application Number 08/807,522, filed February 25, 1997.

FIELD OF THE INVENTION

- 10 This invention relates to newly identified polynucleotides and polypeptides, and their production and uses, as well as their variants, agonists and antagonists, and their uses. In particular, in these and in other regards, the invention relates to novel polynucleotides and polypeptides of the secA (ATPase subunit of preprotein translocase) family, hereinafter referred to as "secA2".

15 BACKGROUND OF THE INVENTION

- It is particularly preferred to employ Staphylococcal genes and gene products as targets for the development of antibiotics. The Staphylococci make up a medically important genera of microbes. They are known to produce two types of disease, invasive and toxigenic. Invasive infections are characterized generally by abscess formation effecting both skin surfaces and deep tissues. *S. aureus* is the second leading cause of bacteremia in cancer patients. Osteomyelitis, septic arthritis, septic thrombophlebitis and acute bacterial endocarditis are also relatively common. There are at least three clinical conditions resulting from the toxigenic properties of Staphylococci. The manifestation of these diseases result from the actions of exotoxins as opposed to tissue invasion and bacteremia. These conditions include: Staphylococcal food poisoning, scalded skin syndrome and toxic shock syndrome

- 25 The frequency of *Staphylococcus aureus* infections has risen dramatically in the past 20 years. This has been attributed to the emergence of multiply antibiotic resistant strains and an increasing population of people with weakened immune systems. It is no longer uncommon to isolate *Staphylococcus aureus* strains which are resistant to some or all of the standard antibiotics. This has created a demand for both new anti-microbial agents and diagnostic tests for this organism.

- 30 SecA is an essential component of the bacterial protein secretion apparatus. This enzyme has been found in a wide variety of Gram-negative and Gram-positive bacteria. SecA proteins from different bacterial species are highly homologous and there is no known mammalian homologue making SecA an ideal target for therapeutic intervention. The proposed model of SecA activity (1,2) is based on the fact that SecA binds open-folded preproteins and interacts with a specific receptor complex at the cytoplasmic membrane. ATP hydrolysis then provides the driving force for translocation of the preprotein by a unique mechanism involving transient insertion of SecA across the cytoplasmic membrane. This mechanism of bacterial translocation requires interaction of SecA with itself (SecA is functional as a dimer (3)), ATP (4), preprotein, membrane phospholipids (5,6,7), SecB (5) and the integral membrane components of the bacterial secretion apparatus (8). These numerous interactions provide us with many opportunities to inhibit the activity of this enzyme. From the above discussion we would expect inhibitors of SecA-mediated protein secretion to be broad spectrum and bactericidal without affecting eukaryotic protein secretion. For recent reviews of Sec dependent preprotein translocation see references 9 and 10.

- 45 Information on this translocation process and SecA can be found for example in: 1. Economou, A. and Wickner, W. (1994) Cell 78, 835-843; 2. Economou, A. et al. (1995) Cell 83, 1171-1181; 3. Driessen, A.J.M. (1993) Biochemistry 32, 13190-13197; 4. van der Wolk, J.P.W. et al. (1995) Journal of Biological Chemistry 270, 18975-18982; 5. Breukink, E. et al. (1995) Journal of Biological Chemistry 270, 7902-7907; 6. Chen, X et al. (1996) Journal of Biological Chemistry 271, 29698-29706; 7. van der Does, C. (1996) Molecular Microbiology 22, 619-629; 8. Snyders, S. et al. (1997) Journal of Biological Chemistry 272, 11302-11306; 9. Wickner, W and Leonard, M.R. (1996) Journal of Biological Chemistry 271, 29514-29516; 10. den Blaauwen, T. and Driessen, J.M. (1996) Arch Microbiol 165, 1-8; 11. WPI Accession No: 96-425087, WO 9626276 A; and 12. WPI Accession No: 95-190181, JP 7107981 A.

- 50 Clearly, there is a need for factors, such as the novel compounds of the invention, that have a present benefit of being useful to screen compounds for antibiotic activity. Such factors are also useful to determine their role in pathogenesis of infection, dysfunction and disease. There is also a need for identification and characterization of such factors and their antagonists and agonists which can play a role in preventing, ameliorating or correcting infections, dysfunctions or diseases.

- 55 The polypeptides of the invention have amino acid sequence homology to a known *Bacillus subtilis* secA protein.

SUMMARY OF THE INVENTION

It is an object of the invention to provide polypeptides that have been identified as novel secA2 polypeptides by
homology between the amino acid sequence set out in Table 1 [SEQ ID NO: 2] and a known amino acid sequence or
sequences of other proteins such as *Bacillus subtilis* secA protein.

It is a further object of the invention to provide polynucleotides that encode secA2 polypeptides, particularly poly-
nucleotides that encode the polypeptide herein designated secA2.

In a particularly preferred embodiment of the invention the polynucleotide comprises a region encoding secA2
polypeptides comprising the sequence set out in Table 1 [SEQ ID NO:1] which includes a full length gene, or a variant
thereof.

In another particularly preferred embodiment of the invention there is a novel secA2 protein from *Staphylococcus*
aureus comprising the amino acid sequence of Table 1 [SEQ ID NO:2], or a variant thereof.

In accordance with another aspect of the invention there is provided an isolated nucleic acid molecule encoding
a mature polypeptide expressible by the *Staphylococcus aureus* WCUH 29 strain contained in the deposited strain.

A further aspect of the invention there are provided isolated nucleic acid molecules encoding secA2, particularly
Staphylococcus aureus secA2, including mRNAs, cDNAs, genomic DNAs. Further embodiments of the invention in-
clude biologically, diagnostically, prophylactically, clinically or therapeutically useful variants thereof, and compositions
comprising the same.

In accordance with another aspect of the invention, there is provided the use of a polynucleotide of the invention
for therapeutic or prophylactic purposes, in particular genetic immunization. Among the particularly preferred embod-
iments of the invention are naturally occurring allelic variants of secA2 and polypeptides encoded thereby.

Another aspect of the invention there are provided novel polypeptides of *Staphylococcus aureus* referred to herein
as secA2 as well as biologically, diagnostically, prophylactically, clinically or therapeutically useful variants thereof, and
compositions comprising the same.

Among the particularly preferred embodiments of the invention are variants of secA2 polypeptide encoded by
naturally occurring alleles of the secA2 gene.

In a preferred embodiment of the invention there are provided methods for producing the aforementioned secA2
polypeptides.

In accordance with yet another aspect of the invention, there are provided inhibitors to such polypeptides, useful
as antibacterial agents, including, for example, antibodies.

In accordance with certain preferred embodiments of the invention, there are provided products, compositions and
methods for assessing secA2 expression, treating disease, for example, disease, such as, infections of the upper
respiratory tract (e.g., otitis media, bacterial tracheitis, acute epiglottitis, thyroiditis), lower respiratory (e.g., empyema,
lung abscess), cardiac (e.g., infective endocarditis), gastrointestinal (e.g., secretory diarrhoea, splenic abscess, retro-
peritoneal abscess), CNS (e.g., cerebral abscess), eye (e.g., blepharitis, conjunctivitis, keratitis, endophthalmitis,
preseptal and orbital cellulitis, dacryocystitis), kidney and urinary tract (e.g., epididymitis, intrarenal and perinephric
abscess, toxic shock syndrome), skin (e.g., impetigo, folliculitis, cutaneous abscesses, cellulitis, wound infection, bac-
terial myositis) bone and joint (e.g., septic arthritis, osteomyelitis), assaying genetic variation, and administering a
secA2 polypeptide or polynucleotide to an organism to raise an immunological response against a bacteria, especially
a *Staphylococcus aureus* bacteria.

In accordance with certain preferred embodiments of this and other aspects of the invention there are provided
polynucleotides that hybridize to secA2 polynucleotide sequences, particularly under stringent conditions.

In certain preferred embodiments of the invention there are provided antibodies against secA2 polypeptides.

In other embodiments of the invention there are provided methods for identifying compounds which bind to or
otherwise interact with and inhibit or activate an activity of a polypeptide or polynucleotide of the invention comprising:
contacting a polypeptide or polynucleotide of the invention with a compound to be screened under conditions to permit
binding to or other interaction between the compound and the polypeptide or polynucleotide to assess the binding to
or other interaction with the compound, such binding or interaction being associated with a second component capable
of providing a detectable signal in response to the binding or interaction of the polypeptide or polynucleotide with the
compound; and determining whether the compound binds to or otherwise interacts with and activates or inhibits an
activity of the polypeptide or polynucleotide by detecting the presence or absence of a signal generated from the binding
or interaction of the compound with the polypeptide or polynucleotide.

In accordance with yet another aspect of the invention, there are provided secA2 agonists and antagonists, pref-
erably bacteriostatic or bacteriocidal agonists and antagonists.

In a further aspect of the invention there are provided compositions comprising a secA2 polynucleotide or a secA2
polypeptide for administration to a cell or to a multicellular organism.

Various changes and modifications within the spirit and scope of the disclosed invention will become readily ap-
parent to those skilled in the art from reading the following descriptions and from reading the other parts of the present

disclosure.

GLOSSARY

5 The following definitions are provided to facilitate understanding of certain terms used frequently herein.
 "Host cell" is a cell which has been transformed or transfected, or is capable of transformation or transfection by an exogenous polynucleotide sequence.

"Identity," as known in the art, is a relationship between two or more polypeptide sequences or two or more polynucleotide sequences, as determined by comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between polypeptide or polynucleotide sequences, as the case may be, as determined by the match between strings of such sequences. "Identity" and "similarity" can be readily calculated by known methods, including but not limited to those described in (*Computational Molecular Biology*, Lesk, A.M., ed., Oxford University Press, New York, 1988; *Biocomputing. Informatics and Genome Projects*, Smith, D.W., ed., Academic Press, New York, 1993; *Computer Analysis of Sequence Data*, Part I, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; *Sequence Analysis in Molecular Biology*, von Heinje, G., Academic Press, 1987; and *Sequence Analysis Primer*, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; and Carillo, H., and Lipman, D., *SIAM J. Applied Math.*, 48: 1073 (1988)). Preferred methods to determine identity are designed to give the largest match between the sequences tested. Methods to determine identity and similarity are codified in publicly available computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, the GCG program package (Devereux, J., et al., *Nucleic Acids Research* 12(1): 387 (1984)), BLASTP, BLASTN, and FASTA (Atschul, S.F. et al., *J. Molec. Biol.* 215: 403-410 (1990)). The BLAST X program is publicly available from NCBI and other sources (*BLAST Manual*, Altschul, S., et al., NCBI NLM NIH Bethesda, MD 20894; Altschul, S., et al., *J. Mol. Biol.* 215: 403-410 (1990)). As an illustration, by a polynucleotide having a nucleotide sequence having at least, for example, 95% "identity" to a reference nucleotide sequence of SEQ ID NO: 1 it is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence of SEQ ID NO: 1. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. These mutations of the reference sequence may occur at the 5 or 3 terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence. Analogously, by a polypeptide having an amino acid sequence having at least, for example, 95% identity to a reference amino acid sequence of SEQ ID NO:2 is intended that the amino acid sequence of the polypeptide is identical to the reference sequence except that the polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the reference amino acid of SEQ ID NO: 2. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a reference amino acid sequence, up to 5% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 5% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

"Isolated" means altered "by the hand of man" from its natural state, i.e., if it occurs in nature, it has been changed or removed from its original environment, or both. For example, a polynucleotide or a polypeptide naturally present in a living organism is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein.

"Polynucleotide(s)" generally refers to any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. "Polynucleotide(s)" include, without limitation, single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions or single-, double- and triple-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded, or triple-stranded regions, or a mixture of single- and double-stranded regions. In addition, "polynucleotide" as used herein refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The strands in such regions may be from the same molecule or from different molecules. The regions may include all of one or more of the molecules, but more typically involve only a region of some of the molecules. One of the molecules of a triple-helical region often is an oligonucleotide. As used herein, the term "polynucleotide(s)" also includes DNAs or RNAs as described above that contain one or more modified bases. Thus, DNAs or RNAs with backbones modified for stability or for other reasons are "polynucleotide(s)" as that term is intended herein. Moreover, DNAs or RNAs comprising unusual bases, such as inosine, or modified

bases, such as tritylated bases, to name just two examples, are polynucleotides as the term is used herein. It will be appreciated that a great variety of modifications have been made to DNA and RNA that serve many useful purposes known to those of skill in the art. The term "polynucleotide(s)" as it is employed herein embraces such chemically, enzymatically or metabolically modified forms of polynucleotides, as well as the chemical forms of DNA and RNA characteristic of viruses and cells, including, for example, simple and complex cells. "Polynucleotide(s)" also embraces short polynucleotides often referred to as oligonucleotide(s).

"Polypeptide(s)" refers to any peptide or protein comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds. "Polypeptide(s)" refers to both short chains, commonly referred to as peptides, oligopeptides and oligomers and to longer chains generally referred to as proteins. Polypeptides may contain amino acids other than the 20 gene encoded amino acids. "Polypeptide(s)" include those modified either by natural processes, such as processing and other post-translational modifications, but also by chemical modification techniques. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature, and they are well known to those of skill in the art. It will be appreciated that the same type of modification may be present in the same or varying degree at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains, and the amino or carboxyl termini. Modifications include, for example, acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, glycosylation, lipid attachment, sulfation, gamma-carboxylation of glutamic acid residues, hydroxylation and ADP-ribosylation, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins, such as arginylation, and ubiquitination. See, for instance, *PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES*, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993) and Wold, F., *Posttranslational Protein Modifications: Perspectives and Prospects*, pgs. 1-12 in *POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS*, B. C. Johnson, Ed., Academic Press, New York (1983); Seifter et al., *Meth. Enzymol.* 182:626-646 (1990) and Rattan et al., *Protein Synthesis: Posttranslational Modifications and Aging*, Ann. N.Y. Acad. Sci. 663: 48-62 (1992). Polypeptides may be branched or cyclic, with or without branching. Cyclic, branched and branched circular polypeptides may result from post-translational natural processes and may be made by entirely synthetic methods, as well.

"Variant(s)" as the term is used herein, is a polynucleotide or polypeptide that differs from a reference polynucleotide or polypeptide respectively, but retains essential properties. A typical variant of a polynucleotide differs in nucleotide sequence from another, reference polynucleotide. Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Nucleotide changes may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below. A typical variant of a polypeptide differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, deletions in any combination. A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. A variant of a polynucleotide or polypeptide may be a naturally occurring such as an allelic variant, or it may be a variant that is not known to occur naturally. Non-naturally occurring variants of polynucleotides and polypeptides may be made by mutagenesis techniques, by direct synthesis, and by other recombinant methods known to skilled artisans.

DESCRIPTION OF THE INVENTION

The invention relates to novel secA2 polypeptides and polynucleotides as described in greater detail below. In particular, the invention relates to polypeptides and polynucleotides of a novel secA2 of *Staphylococcus aureus*, which is related by amino acid sequence homology to *Bacillus subtilis* secA polypeptide. The invention relates especially to secA2 having the nucleotide and amino acid sequences set out in Table 1 [SEQ ID NO: 1] and Table 1 [SEQ ID NO: 2] respectively, and to the secA2 nucleotide sequences of the DNA in the deposited strain and amino acid sequences encoded thereby.

TABLE 1

5 secA2 Polynucleotide and Polypeptide Sequences

(A) Sequences from *Staphylococcus aureus* secA2 polynucleotide sequence [SEQ ID
10 NO:1].

5'-ATGAAACATAAACTAGATGTTACGATAAACGAATTAAGACTAAAGTCGAT
15 TAGAAAAATAGTGAAACGCATCAATACATGGAGCGATGAAGTTAAATCAT
ATTCTGATGATGCGTTAAAACAAAAGACAATAGAATTTAAAGAACGTTTA
GCATCAGGAGTTGATACACTAGATACATTGTTACCTGAAGCTTATGCAGT
GGCACGTGAAGCGAGCTGGAGAGTATTAGGTATGTATCCTAAAGAAGTTC
20 AGTTAATCGGAGCGATTGTATTGCATGAAGGTAACATTGCGGAGATGCAA
ACAGGCGAAGGTAAAACATTAACGGCAACGATGCCTTTATATTTAAATGC
TCTTTCGGGAAAAGGAACATATTTAATTACAACGAATGATTATTTAGCGA
25 AGCGAGATTTTGAAGAGATGCAACCATTATATGAATGGTTAGGTTTAACT
GCTTCATTAGGTTTTGTGTGATATTGTGGATTATGAGTATCAAAAAGGGGA
AAAACGTAATATATATGAACATGATATTATATATACTAATGGACGTC
TCGGTTTTGATTATTTAATTGATAATTTGGCTGATAGTGC GGAAGGGAAA
30 TTTTACCACAATTAAATTACGGTATTATTGATGAAGTGGATTCAATCAT
TTTAGATGCTGCTCAAACACCATTAGTTATTTTCGGGTGCACCAAGATTAC
AATCAAATCTATTTTCATATTGTGAAAGAGTTTGTAGATACATTGATTGAA
GACGTGCATTTTAAATGAAGAAGACCAAAAAGAAATATGGCTGTTAAA
35 TCAAGGTATTGAAGCGGCACAATCATACTTTAATGTTGAAGATTTATATA
GCGAACAAGCGATGGTCCTAGTGCCTAATATTAATTTAGCACTGCGCGCA
CAATATTTGTTTGAATCTAATGTGCGATTACTTTGTATATAATGGTGATAT
40 TGTTTTAATTGACCGTATTACAGGTCGTATGTTACCGGGAAGTTC
AAGCTGGACTTCACCAAGCTATTGAAGCGAAAGAAGGTATGGAGGTTTCA

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TDKSVMATITFQNLFKLFESFSGMTATGKLGSEFFDLYSKIVVQAPTDK
 AIQRIDEPPDKVFRSVDEKNIAMIHDIHELHETGRPVLLITRTAEAAEYFS
 5 EVLFQMDIPNNLLIAQNVAKEAQMIAEAGQIGSMTVATSMAGRGTDIKLG
 EGVEALGGLAVIIHEHMENSRVDRQLRGRSGRQGDPGSSCIYISLDDYLV
 KRWSDSNLAENNQLYSLDAQRLSQSNLFRKVKQIVVKAQRISSEEQGVKA
 10 REMANEFEKSSISIQRDLVYEERNRVLEIDDAENRDFKALAKDVFEMFVNE
 EKVLTKSRVVEYIYQNLQFQNKDVACVNFKDKQAVVTFLLEQFEKQLAL
 NRKNMQSAYYYNIFVQKVFLKAIDSCWLEQVDYLOQLKASVNQRQNGQRN
 AIFEYHRVALDSFEVMTRNIKKRMVKNICQSMITFDKEGMPVIHFP-COOH
 15

(C) Polynucleotide sequence embodiments [SEQ ID NO:1].

X-(R₁)_n-ATGAAACATAAACTAGATGTTACGATAAACGAATTAAGACTAAAGTCGAT
 20 TAGAAAAATAGTGAAACGCATCAATACATGGAGCGATGAAGTTAAATCAT
 ATTCTGATGATGCGTTAAAAACAAAAGACAATAGAATTTAAAGAACGTTTA
 GCATCAGGAGTTGATACACTAGATACATTGTTACCTGAAGCTTATGCAGT
 GGCACGTGAAGCGAGCTGGAGAGTATTAGGTATGTATCCTAAAGAAGTTC
 25 AGTTAATCGGAGCGATTGTATTGCATGAAGGTAACATTGCGGAGATGCAA
 ACAGGCGAAGGTAAACATTAACGGCAACGATGCCTTTATATTTAAATGC
 TCTTTCTGGGAAAAGGAACATATTTAATTACAACGAATGATTATTTAGCGA
 30 AGCGAGATTTTGAAGAGATGCAACCATTATATGAATGGTTAGGTTTAACT
 GCTTCATTAGGTTTTGTGATATTGTGGATTATGAGTATCAAAAAGGGGA
 AAAACGTAATATATATGAACATGATATTATATATACAACCTAATGGACGTC
 TCGGTTTTGATTATTTAATTGATAATTTGGCTGATAGTGCGGAAGGGAAA
 35 TTTTTACCACAATTAAATTACGGTATTATTGATGAAGTGGATTCAATCAT
 TTTAGATGCTGCTCAAACACCATTAGTTATTTCTGGGTGCACCAAGATTAC
 AATCAAATCTATTTTCATATTGTGAAAAGAGTTTGTAGATACATTGATTGAA
 40 GACGTGCATTTTAAAATGAAGAAGACCAAAAAAGAAATATGGCTGTAAA
 TCAAGGTATTGAAGCGGCACAATCATACTTTAATGTTGAAGATTTATATA
 GCGAACAAGCGATGGTCCTAGTGCGTAATATTAATTTAGCACTGCGCGCA
 CAATATTTGTTTGAATCTAATGTGCGATTACTTTGTATATAATGGTGATAT
 45 TGTTTTAATTGACCGTATTACAGGTGCTATGTTACCGGGAACCTAAGTTGC
 AAGCTGGACTTCACCAAGCTATTGAAGCGAAAGAAGGTATGGAGGTTTCA
 ACAGATAAAAGTGTTATGGCAACAATTACCTTCCAGAATTTATTTAACT
 50 TTTTGAATCATTTTCAGGTATGACAGCTACAGGAAAATTAGGCGAATCAG
 AGTTCCTTTGATTTGTATTCAAAAATAGTCGTACAAGCACCAACTGATAAA
 GCGATTCAACGTATCGATGAACCAGATAAAGTGTTTCGTTTCAGTTGATGA
 GAAAAACATCGCGATGATTCATGATATAGTTGAACTTCATGAAACGGGGC
 55

GACCGGTTTTACTCATAACGAGAACTGCTGAAGCGGCTGAATACTTTTCC
 GAAGTATTATTCCAAATGGATATTCCTAATAATTTACTCATTGCGCAAAA
 5 TGTGCAAAAGAAGCGCAGATGATAGCTGAAGCAGGGCAAATTGGTTCCA
 TGACTGTTGCGACTAGTATGGCAGGTCGAGGCACAGATATTAACTTGGT
 GAAGGTGTCGAAGCATTAGGTGGATTAGCTGTTATTATTTCATGAACATAT
 10 GGAAAATAGCCGTGTAGACAGGCAATTACGTGGTCGTTCTGGTAGACAAG
 GGGATCCGGGATCATCTTGTATATATATTTCACTAGATGATTATTTAGTT
 AAGCGATGGAGCGATAGTAATTTAGCGGAAAATAATCAATTATATTCATT
 AGATGCACAACGATTATCGCAAAGTAATTTGTTTAATCGCAAAGTTAAGC
 15 AAATTGTAGTTAAAGCGCAGCGTATCTCGGAAGAACAAGGGGTTAAAGCT
 CGTGAATGGCTAATGAATTTGAAAAAGCATTAGTATACAGCGAGATCT
 TGTATACGAGGAACGCAATCGAGTTTTAGAAATAGATGATGCTGAGAATC
 GAGATTTTAAAGCGTTAGCTAAAGATGTATTTGAAATGTTTGTAATGAG
 20 GAAAAGGTGCTAACAAAATCGCGTGTGTGCGAGTATATTTATCAAAATTT
 AAGTTTCCAATTTAATAAAGATGTGGCTTGTGTTAATTTTAAAGATAAGC
 AAGCAGTAGTGACATTTTTATTAGAGCAATTTGAAAAGCAATTAGCTTTG
 25 AATCGTAAAAACATGCAAAGTGCATATTATTATAATATTTTCGTTCAAAA
 AGTCTTTTTGAAAGCAATTGATTCATGTTGGTTAGAACAAGTCGACTATT
 TACAACAATTTAAAGCAAGCGTTAATCAACGACAAAATGGGCAACGTAAC
 GCTATTTTTGAATACCATAGAGTTGCATTAGATTCATTTGAAGTTATGAC
 30 ACGAAATATCAAAAAAGAATGGTGAAAAATATTTGTCAAAGCATGATTA
 CATTTGATAAAGAGGGTATGCCAGTTATACATTTTCCA- (R₂)_n-Y

(D) Polypeptide sequence embodiments [SEQ ID NO:2].

X- (R₁)_n-MKHKLDVTINELRLKSIRKIVKRINTWSDEVKSYSDALKQKTIEFKERL
 ASGVDTLDTLLPEAYAVAREASWRVLGMYPKVQLIGAIVLHEGNIAEMQ
 40 TGEGKTLTATMPLYLNALSGKGYLITNDYLAKRDFEEMQPLYEWLGLT
 ASLGFVDIVDYEYQKGEKRNIYEHDIIYTTNGRLGFDYLDNLADSAEGK
 FLPQLNYGIIDEVDSIILDAAQTPLVISGAPRLQSNLFHIVKEFVDTLIE
 45 DVHFKMKKTKKEIWLLNQIEAAQSYFNVEDLYSEQAMVLVRNINLALRA
 QYLFESNVDFVYNGDIVLIDRITGRMLPGTKLQAGLHQAIEAKEGMEVS
 TDKSVMATITFQNLFKLFESFGMTATGKLGESEFFDLYSKIIVVQAPTDK
 AIQRIDEPDKVFRSVDEKNIAMIHDIIVELHETGRPVLLITRTAEAAEYFS
 50 EVLFQMDIPNNLLIAQNVAKEAQMIAEAGQIGSMTVATSMAGRGTDIKLG
 EGVEALGGLAVIIHEHMENSRVDRQLRGRSGRQGDGSSCIYISLDDYLV
 KRWSDSNLAENNQLYSLDAQRLSQSNLFNRKVKQIVVKAQRISEEQGVKA
 55 REMANEFEKSISIQRDLVYEERNRVLEIDDAENRDFKALAKDVFEMFVNE

EKVLTKSRVVEYIYQNLQFNKDVACVNFKDKQAVVTFLLEQFEKQLAL
 NRKNMQSAYYYNIFVQKVLKAIDSCWLEQVDYLQQLKASVNQRQNGQRN
 5 AIFEYHRVALDSFEVMTRNIKKRMVKNICQSMITFDKEGMPVIHFP- $(R_2)_n-Y$

(E) Sequences from *Staphylococcus aureus* secA2 polynucleotide ORF sequence [SEQ ID NO:3].

5'-1 GTTAATCAAG TATCGAAGCG GAACAATCAT ACTTTAATGT TGAAGATTTA
 51 TATNGCGAAC AAGCGATGGT CCTAGTGCCT AATATTAATT TAGCACTGCG
 15 101 CGCACAATAT TTGTTNGNAT CTNATGTCGA TTACTTTGTA TATNNTGGTG
 151 ATATTGTTTT AACTGACCNC ATTACAGGTC GTNTGTTACC GGNAACTAAG
 20 201 TTGCAAGCTG GACTTCACCA NGCTATTGAA GCGAAAGAAG GTATGGAGGT
 251 TTCAACAGAT AAAAGTGTTA TGCCAACCAA TTACCCTTCC AGAATTTATT
 301 TAAACTTTTT GAATCAATTT TCAGGTATGA CAAGCTACAG GAAAATTAGG
 25 351 CGAATCAGAG TTCTTTGATT TGTATTCANA AATAGTCGTA CAAGCACCCA
 401 ACTGATAAAG CGATTCAACG TATCGATGAA CCAGATAAAG TGTTTCGTTC
 451 AGTTGATGAG AAAAACATCG CGATGATTCA TTGATATAGT TGAACTTCAT
 30 501 GANNCGGGGC CGACCGGTTT TACCTCATAA CCGAGNACTG CTGAAGCGGC
 551 TTGAATACTT TTCNGAAGTA TTATTCCAAA TGGATATTCC TAATAATTTA
 35 601 CTCATTGCGC AAAATGTTCC AAAAGAAGCG CAGATGATAG CTGAAGCAGG
 651 CCAAATTGGT TCCATGACTG TTGCGACTAG TATGGCAGGT CGAGGCACAG
 40 701 ATATTAAACT TGGTGAAGGT GTCGAAGCAT TAGCTGGATT AGCTGTTATT
 751 ATTCATGAAC ATATGGAAAA TAGCCGTGTA GACAGGCAAT TACGTGGTCG
 801 TTCTGGTAGA CAAGGGGATC CGGGATCATC TTGTATATAT ATTTCACTAG
 45 851 ATGATTATTT AGNTAAGCGA TGGAGCGATA GTAATTTAGC GGAAAATAAT
 901 CAATTATATT CANTAGATGC ACAACGATTA TCGCAAAGTA ATTTGTTTAA
 50 951 TCGNAAAGTT AAGCAAATTG TAGTTAAAGC GCAGCGTATC TCGGAAAGAA
 1001 CAAGGGGTTA AAGCTCGGTG AAATGGCTTA ATTGAATTTG NNAAAAGCA
 55 1051 TNAGTATTCA GCGAAGATCT TNGTATTTAC GANGGAACGC AAATCCGAGT

1101 TTTTAGAAAT TAGATTGATG CTGAGAATCC NAGATTTTTTA ANGCGGTTAG
 5 1151 CTTAAAGATT GTATTTGAAA TNGTTTGGGG NAATGANGGA AANGGTGCTA
 1201 ACAAATCGC GNGTTGGGCG AGTATATTTT ATCAAAAATT TAAGTTNCCA
 10 1251 ATTTAATAAA GATGTGGCTT GTGTAAATTT TAAAGATAAG CAAGCAGNAG
 1301 TGACATTTTT ATTAGAGCAA TTTGAAAAGC AATTAGCTTT GGANTCCGTA
 1351 AAAACATGCA ANGNGCATAT TATTATAATA TTNCCGGCCA AAANGTCTTT
 15 1401 NGGGAAAGCA ATTGATNCAA GTTGGGGTTA GGAACAAGTC GGCTTTTNAC
 1451 AACAAATTAA NAGCAAGCGN TAATCAAACG ACAAANTGG CAACCT-3'

(F) secA2 polypeptide sequence deduced from the polynucleotide ORF sequence in this table [SEQ ID NO:4].

NH₂-1 MDIPNNLLIA QNVPKEAQMI AEAGQIGSMT VATSMAGRGT DIKLGEVGEA
 25 51 LAGLAVIIHE HMENSRVDRQ LRGRSGRQGD PGSSCIYISL DDYLXKRWSD
 101 SNLAENNQLY SXDAQRLSQS NLFNRKVQI VVKAQRISER TRG-COOH

Deposited materials

A deposit containing a *Staphylococcus aureus* WCUH 29 strain has been deposited with the National Collections of Industrial and Marine Bacteria Ltd. therein "NCIMB"), 23 St. Machar Drive, Aberdeen AB2 1RY, Scotland on 11 September 1995 and assigned NCIMB Deposit No. 40771, and is referred to as *Staphylococcus aureus* WCUH29 on deposit. The *Staphylococcus aureus* strain deposit is referred to herein as "the deposited strain" or as "the DNA of the deposited strain."

The deposited strain contains the full length secA2 gene. The sequence of the polynucleotides contained in the deposited strain, as well as the amino acid sequence of the polypeptide encoded thereby, are controlling in the event of any conflict with any description of sequences herein.

The deposit of the deposited strain has been made under the terms of the Budapest Treaty on the International Recognition of the Deposit of Micro-organisms for Purposes of Patent Procedure. The strain will be irrevocably and without restriction or condition released to the public upon the issuance of a patent. The deposited strain is provided merely as convenience to those of skill in the art and is not an admission that a deposit is required for enablement, such as that required under 35 U.S.C. §112.

A license may be required to make, use or sell the deposited strain, and compounds derived therefrom, and no such license is hereby granted.

Polypeptides

The polypeptides of the invention include the polypeptide of Table 1 [SEQ ID NO:2] (in particular the mature polypeptide) as well as polypeptides and fragments, particularly those which have the biological activity of secA2, and also those which have at least 70% identity to a polypeptide of Table 1 [SEQ ID NOS:2 and 4] or the relevant portion, preferably at least 80% identity to a polypeptide of Table 1 [SEQ ID NOS:2 and 4], and more preferably at least 90% similarity (more preferably at least 90% identity) to a polypeptide of Table 1 [SEQ ID NOS:2 and 4] and still more preferably at least 95% similarity (still more preferably at least 95% identity) to a polypeptide of Table 1 [SEQ ID NOS:2 and 4] and also include portions of such polypeptides with such portion of the polypeptide generally containing at

least 30 amino acids and more preferably at least 50 amino acids.

The invention also includes polypeptides of the formula set forth in Table 1 (D) [SEQ ID NO:2] wherein, at the amino terminus, X is hydrogen, and at the carboxyl terminus, Y is hydrogen or a metal, R_1 and R_2 is any amino acid residue, and n is an integer between 1 and 1000. Any stretch of amino acid residues denoted by either R group, where R is greater than 1, may be either a heteropolymer or a homopolymer, preferably a heteropolymer.

A fragment is a variant polypeptide having an amino acid sequence that entirely is the same as part but not all of the amino acid sequence of the aforementioned polypeptides. As with secA2 polypeptides fragments may be "free-standing," or comprised within a larger polypeptide of which they form a part or region, most preferably as a single continuous region, a single larger polypeptide.

Preferred fragments include, for example, truncation polypeptides having a portion of an amino acid sequence of Table 1 [SEQ ID NOS:2 and 4], or of variants thereof, such as a continuous series of residues that includes the amino terminus, or a continuous series of residues that includes the carboxyl terminus. Degradation forms of the polypeptides of the invention in a host cell, particularly a *Staphylococcus aureus*, are also preferred. Further preferred are fragments characterized by structural or functional attributes such as fragments that comprise alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions.

Also preferred are biologically active fragments which are those fragments that mediate activities of secA2, including those with a similar activity or an improved activity, or with a decreased undesirable activity. Also included are those fragments that are antigenic or immunogenic in an animal, especially in a human. Particularly preferred are fragments comprising receptors or domains of enzymes that confer a function essential for viability of *Staphylococcus aureus* or the ability to initiate, or maintain cause disease in an individual, particularly a human.

Variants that are fragments of the polypeptides of the invention may be employed for producing the corresponding full-length polypeptide by peptide synthesis; therefore, these variants may be employed as intermediates for producing the full-length polypeptides of the invention.

Polynucleotides

Another aspect of the invention relates to isolated polynucleotides, including the full length gene, that encode the secA2 polypeptide having a deduced amino acid sequence of Table 1 [SEQ ID NOS:2 and 4] and polynucleotides closely related thereto and variants thereof.

Using the information provided herein, such as a polynucleotide sequence set out in Table 1 [SEQ ID NOS:1 and 3], a polynucleotide of the invention encoding secA2 polypeptide may be obtained using standard cloning and screening methods, such as those for cloning and sequencing chromosomal DNA fragments from bacteria using *Staphylococcus aureus* WCUH 29 cells as starting material, followed by obtaining a full length clone. For example, to obtain a polynucleotide sequence of the invention, such as a sequence given in Table 1 [SEQ ID NOS:1 and 3], typically a library of clones of chromosomal DNA of *Staphylococcus aureus* WCUH 29 in *E. coli* or some other suitable host is probed with a radiolabeled oligonucleotide, preferably a 17-mer or longer, derived from a partial sequence. Clones carrying DNA identical to that of the probe can then be distinguished using stringent conditions. By sequencing the individual clones thus identified with sequencing primers designed from the original sequence it is then possible to extend the sequence in both directions to determine the full gene sequence. Conveniently, such sequencing is performed using denatured double stranded DNA prepared from a plasmid clone. Suitable techniques are described by Maniatis, T., Fritsch, E.F. and Sambrook et al., *MOLECULAR CLONING, A LABORATORY MANUAL*, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989). (see in particular Screening By Hybridization 1.90 and Sequencing Denatured Double-Stranded DNA Templates 13.70). Illustrative of the invention, the polynucleotide set out in Table 1 [SEQ ID NO:1] was discovered in a DNA library derived from *Staphylococcus aureus* WCUH 29.

The DNA sequence set out in Table 1 [SEQ ID NOS:1] contains an open reading frame encoding a protein having about the number of amino acid residues set forth in Table 1 [SEQ ID NO:2] with a deduced molecular weight that can be calculated using amino acid residue molecular weight values well known in the art. The polynucleotide of SEQ ID NO: 1, between nucleotide number 1 through number 2388 encodes the polypeptide of SEQ ID NO:2. The stop codon begins at nucleotide number 2389 of SEQ ID NO:1.12388

The secA2 protein of the invention is structurally related to other proteins of the secA (ATPase subunit of preprotein translocase) family, as shown by the results of sequencing the DNA encoding secA2 of the deposited strain. The protein exhibits greatest homology to *Bacillus subtilis* secA protein among known proteins. The secA2 protein of Table 1 [SEQ ID NO:2] has about 39% identity over its entire length and about 62% similarity over its entire length with the amino acid sequence of *Bacillus subtilis* secA polypeptide.

The invention provides a polynucleotide sequence identical over its entire length to the coding sequence in Table 1 [SEQ ID NO:1]. Also provided by the invention is the coding sequence for the mature polypeptide or a fragment

thereof, by itself as well as the coding sequence for the mature polypeptide or a fragment in reading frame with other coding sequence, such as those encoding a leader or secretory sequence, a pre-, or pro- or prepro- protein sequence. The polynucleotide may also contain non-coding sequences, including for example, but not limited to non-coding 5' and 3' sequences, such as the transcribed, non-translated sequences, termination signals, ribosome binding sites, sequences that stabilize mRNA, introns, polyadenylation signals, and additional coding sequence which encode additional amino acids. For example, a marker sequence that facilitates purification of the fused polypeptide can be encoded. In certain embodiments of the invention, the marker sequence is a hexa-histidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Gentz *et al.*, *Proc. Natl. Acad. Sci., USA* 86: 821-824 (1989), or an HA tag (Wilson *et al.*, *Cell* 37: 767 (1984). Polynucleotides of the invention also include, but are not limited to, polynucleotides comprising a structural gene and its naturally associated sequences that control gene expression.

A preferred embodiment of the invention is a polynucleotide of comprising nucleotide 1 to 2388 or 2389 set forth in SEQ ID NO: 1 of Table 1 which encode the secA2 polypeptide.

The invention also includes polynucleotides of the formula set forth in Table 1 (C)[SEQ ID NO:1] wherein, at the 5' end of the molecule, X is hydrogen, and at the 3' end of the molecule, Y is hydrogen or a metal, R₁ and R₂ is any nucleic acid residue, and n is an integer between 1 and 1000. Any stretch of nucleic acid residues denoted by either R group, where R is greater than 1, may be either a heteropolymer or a homopolymer, preferably a heteropolymer.

The term "polynucleotide encoding a polypeptide" as used herein encompasses polynucleotides that include a sequence encoding a polypeptide of the invention, particularly a bacterial polypeptide and more particularly a polypeptide of the *Staphylococcus aureus* secA2 having the amino acid sequence set out in Table 1 [SEQ ID NO:2]. The term also encompasses polynucleotides that include a single continuous region or discontinuous regions encoding the polypeptide (for example, interrupted by integrated phage or an insertion sequence or editing) together with additional regions, that also may contain coding and/or non-coding sequences.

The invention further relates to variants of the polynucleotides described herein that encode for variants of the polypeptide having the deduced amino acid sequence of Table 1 [SEQ ID NO:2]. Variants that are fragments of the polynucleotides of the invention may be used to synthesize full-length polynucleotides of the invention.

Further particularly preferred embodiments are polynucleotides encoding secA2 variants, that have the amino acid sequence of secA2 polypeptide of Table 1 [SEQ ID NO:2] in which several, a few, 5 to 10, 1 to 5, 1 to 3, 2, 1 or no amino acid residues are substituted, deleted or added, in any combination. Especially preferred among these are silent substitutions, additions and deletions, that do not alter the properties and activities of secA2.

Further preferred embodiments of the invention are polynucleotides that are at least 70% identical over their entire length to a polynucleotide encoding secA2 polypeptide having an amino acid sequence set out in Table 1 [SEQ ID NOS:2 and 4], and polynucleotides that are complementary to such polynucleotides. Alternatively, most highly preferred are polynucleotides that comprise a region that is at least 80% identical over its entire length to a polynucleotide encoding secA2 polypeptide of the deposited strain and polynucleotides complementary thereto. In this regard, polynucleotides at least 90% identical over their entire length to the same are particularly preferred, and among these particularly preferred polynucleotides, those with at least 95% are especially preferred. Furthermore, those with at least 97% are highly preferred among those with at least 95%, and among these those with at least 98% and at least 99% are particularly highly preferred, with at least 99% being the more preferred.

Preferred embodiments are polynucleotides that encode polypeptides that retain substantially the same biological function or activity as the mature polypeptide encoded by the DNA of Table 1 [SEQ ID NO:1].

The invention further relates to polynucleotides that hybridize to the herein above-described sequences. In this regard, the invention especially relates to polynucleotides that hybridize under stringent conditions to the herein above-described polynucleotides. As herein used, the terms "stringent conditions" and "stringent hybridization conditions" mean hybridization will occur only if there is at least 95% and preferably at least 97% identity between the sequences. An example of stringent hybridization conditions is overnight incubation at 42°C in a solution comprising: 50% formamide, 5x SSC (150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 micrograms/ml denatured, sheared salmon sperm DNA, followed by washing the hybridization support in 0.1x SSC at about 65°C. Hybridization and wash conditions are well known and exemplified in Sambrook, *et al.*, *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor, N.Y., (1989), particularly Chapter 11 therein.

The invention also provides a polynucleotide consisting essentially of a polynucleotide sequence obtainable by screening an appropriate library containing the complete gene for a polynucleotide sequence set forth in SEQ ID NO: 1 or SEQ ID NO:3 under stringent hybridization conditions with a probe having the sequence of said polynucleotide sequence set forth in SEQ ID NO:1 or a fragment thereof; and isolating said DNA sequence. Fragments useful for obtaining such a polynucleotide include, for example, probes and primers described elsewhere herein.

As discussed additionally herein regarding polynucleotide assays of the invention, for instance, polynucleotides of the invention as discussed above, may be used as a hybridization probe for RNA, cDNA and genomic DNA to isolate full-length cDNAs and genomic clones encoding secA2 and to isolate cDNA and genomic clones of other genes that

have a high sequence similarity to the *secA2* gene. Such probes generally will comprise at least 15 bases. Preferably, such probes will have at least 30 bases and may have at least 50 bases. Particularly preferred probes will have at least 30 bases and will have 50 bases or less.

For example, the coding region of the *secA2* gene may be isolated by screening using the DNA sequence provided in SEQ ID NO: 1 to synthesize an oligonucleotide probe. A labeled oligonucleotide having a sequence complementary to that of a gene of the invention is then used to screen a library of cDNA, genomic DNA or mRNA to determine which members of the library the probe hybridizes to.

The polynucleotides and polypeptides of the invention may be employed, for example, as research reagents and materials for discovery of treatments of and diagnostics for disease, particularly human disease, as further discussed herein relating to polynucleotide assays.

Polynucleotides of the invention that are oligonucleotides derived from the sequences of SEQ ID NOS:1 and/or 2 may be used in the processes herein as described, but preferably for PCR, to determine whether or not the polynucleotides identified herein in whole or in part are transcribed in bacteria in infected tissue. It is recognized that such sequences will also have utility in diagnosis of the stage of infection and type of infection the pathogen has attained.

The invention also provides polynucleotides that may encode a polypeptide that is the mature protein plus additional amino or carboxyl-terminal amino acids, or amino acids interior to the mature polypeptide (when the mature form has more than one polypeptide chain, for instance). Such sequences may play a role in processing of a protein from precursor to a mature form, may allow protein transport, may lengthen or shorten protein half-life or may facilitate manipulation of a protein for assay or production, among other things. As generally is the case *in vivo*, the additional amino acids may be processed away from the mature protein by cellular enzymes.

A precursor protein, having the mature form of the polypeptide fused to one or more prosequences may be an inactive form of the polypeptide. When prosequences are removed such inactive precursors generally are activated. Some or all of the prosequences may be removed before activation. Generally, such precursors are called proproteins.

In sum, a polynucleotide of the invention may encode a mature protein, a mature protein plus a leader sequence (which may be referred to as a preprotein), a precursor of a mature protein having one or more prosequences that are not the leader sequences of a preprotein, or a preproprotein, which is a precursor to a proprotein, having a leader sequence and one or more prosequences, which generally are removed during processing steps that produce active and mature forms of the polypeptide.

30 Vectors, host cells, expression

The invention also relates to vectors that comprise a polynucleotide or polynucleotides of the invention, host cells that are genetically engineered with vectors of the invention and the production of polypeptides of the invention by recombinant techniques. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the invention.

For recombinant production, host cells can be genetically engineered to incorporate expression systems or portions thereof or polynucleotides of the invention. Introduction of a polynucleotide into the host cell can be effected by methods described in many standard laboratory manuals, such as Davis et al., *BASIC METHODS IN MOLECULAR BIOLOGY*, (1986) and Sambrook et al., *MOLECULAR CLONING: A LABORATORY MANUAL*, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989), such as, calcium phosphate transfection, DEAE-dextran mediated transfection, transfection, microinjection, cationic lipid-mediated transfection, electroporation, transduction, scrape loading, ballistic introduction and infection.

Representative examples of appropriate hosts include bacterial cells, such as streptococci, staphylococci, enterococci *E. coli*, streptomyces and *Bacillus subtilis* cells; fungal cells, such as yeast cells and *Aspergillus* cells; insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells such as CHO, COS, HeLa, C127, 3T3, BHK, 293 and Bowes melanoma cells; and plant cells.

A great variety of expression systems can be used to produce the polypeptides of the invention. Such vectors include, among others, chromosomal, episomal and virus-derived vectors, *e.g.*, vectors derived from bacterial plasmids, from bacteriophage, from transposons, from yeast episomes, from insertion elements, from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, such as cosmids and phagemids. The expression system constructs may contain control regions that regulate as well as engender expression. Generally, any system or vector suitable to maintain, propagate or express polynucleotides and/or to express a polypeptide in a host may be used for expression in this regard. The appropriate DNA sequence may be inserted into the expression system by any of a variety of well-known and routine techniques, such as, for example, those set forth in Sambrook et al., *MOLECULAR CLONING, A LABORATORY MANUAL*, (*supra*).

For secretion of the translated protein into the lumen of the endoplasmic reticulum, into the periplasmic space or

into the extracellular environment, appropriate secretion signals may be incorporated into the expressed polypeptide. These signals may be endogenous to the polypeptide or they may be heterologous signals.

Polypeptides of the invention can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography, and lectin chromatography. Most preferably, high performance liquid chromatography is employed for purification. Well known techniques for refolding protein may be employed to regenerate active conformation when the polypeptide is denatured during isolation and or purification.

Diagnostic Assays

This invention is also related to the use of the secA2 polynucleotides of the invention for use as diagnostic reagents. Detection of secA2 in a eukaryote, particularly a mammal, and especially a human, will provide a diagnostic method for diagnosis of a disease. Eukaryotes (herein also "individual(s)"), particularly mammals, and especially humans, infected with an organism comprising the secA2 gene may be detected at the nucleic acid level by a variety of techniques.

Nucleic acids for diagnosis may be obtained from an infected individual's cells and tissues, such as bone, blood, muscle, cartilage, and skin. Genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR or other amplification technique prior to analysis. RNA or cDNA may also be used in the same ways. Using amplification, characterization of the species and strain of prokaryote present in an individual, may be made by an analysis of the genotype of the prokaryote gene. Deletions and insertions can be detected by a change in size of the amplified product in comparison to the genotype of a reference sequence. Point mutations can be identified by hybridizing amplified DNA to labeled secA2 polynucleotide sequences. Perfectly matched sequences can be distinguished from mismatched duplexes by RNase digestion or by differences in melting temperatures. DNA sequence differences may also be detected by alterations in the electrophoretic mobility of the DNA fragments in gels, with or without denaturing agents, or by direct DNA sequencing. See, e.g., Myers et al., *Science*, 230: 1242 (1985). Sequence changes at specific locations also may be revealed by nuclease protection assays, such as RNase and S1 protection or a chemical cleavage method. See, e.g., Cotton et al., *Proc. Natl. Acad. Sci., USA*, 85: 4397-4401(1985).

Cells carrying mutations or polymorphisms in the gene of the invention may also be detected at the DNA level by a variety of techniques, to allow for serotyping, for example. For example, RT-PCR can be used to detect mutations. It is particularly preferred to used RT-PCR in conjunction with automated detection systems, such as, for example, GeneScan. RNA or cDNA may also be used for the same purpose, PCR or RT-PCR. As an example, PCR primers complementary to a nucleic acid encoding secA2 can be used to identify and analyze mutations. Examples of representative primers are shown below in Table 2.

Table 2

Primers for amplification of secA2 polynucleotides

<u>SEQ ID NO</u>	<u>PRIMER SEQUENCE</u>
5	5'-ATGAAACATA AACTAGATGT TACG-3'
6	5'-TGGAAATGT ATAAGTGGCA TACC-3'

The invention further provides these primers with 1, 2, 3 or 4 nucleotides removed from the 5' and/or the 3' end. These primers may be used for, among other things, amplifying secA2 DNA isolated from a sample derived from an individual. The primers may be used to amplify the gene isolated from an infected individual such that the gene may then be subject to various techniques for elucidation of the DNA sequence. In this way, mutations in the DNA sequence may be detected and used to diagnose infection and to serotype and/or classify the infectious agent.

The invention further provides a process for diagnosing, disease, preferably bacterial infections, more preferably infections by *Staphylococcus aureus*, and most preferably disease, such as, infections of the upper respiratory tract (e.g., otitis media, bacterial tracheitis, acute epiglottitis, thyroiditis), lower respiratory (e.g., empyema, lung abscess), cardiac (e.g., infective endocarditis), gastrointestinal (e.g., secretory diarrhoea, splenic abscess, retroperitoneal ab-

5 scess), CNS (e.g., cerebral abscess), eye (e.g., blepharitis, conjunctivitis, keratitis, endophthalmitis, preseptal and orbital cellulitis, dacryocystitis), kidney and urinary tract (e.g., epididymitis, intrarenal and perinephric abscess, toxic shock syndrome), skin (e.g., impetigo, folliculitis, cutaneous abscesses, cellulitis, wound infection, bacterial myositis) bone and joint (e.g., septic arthritis, osteomyelitis), comprising determining from a sample derived from an individual
 a increased level of expression of polynucleotide having the sequence of Table 1 [SEQ ID NO: 1]. Increased or decreased expression of secA2 polynucleotide can be measured using any one of the methods well known in the art for the quantitation of polynucleotides, such as, for example, amplification, PCR, RT-PCR, RNase protection, Northern blotting and other hybridization methods.

10 In addition, a diagnostic assay in accordance with the invention for detecting over-expression of secA2 protein compared to normal control tissue samples may be used to detect the presence of an infection, for example. Assay techniques that can be used to determine levels of a secA2 protein, in a sample derived from a host are well-known to those of skill in the art. Such assay methods include radioimmunoassays, competitive-binding assays, Western Blot analysis and ELISA assays.

15 Antibodies

The polypeptides of the invention or variants thereof, or cells expressing them can be used as an immunogen to produce antibodies immunospecific for such polypeptides. "Antibodies" as used herein includes monoclonal and polyclonal antibodies, chimeric, single chain, simianized antibodies and humanized antibodies, as well as Fab fragments, including the products of an Fab immunoglobulin expression library.

20 Antibodies generated against the polypeptides of the invention can be obtained by administering the polypeptides or epitope-bearing fragments, analogues or cells to an animal, preferably a nonhuman, using routine protocols. For preparation of monoclonal antibodies, any technique known in the art that provides antibodies produced by continuous cell line cultures can be used. Examples include various techniques, such as those in Kohler, G. and Milstein, C., *Nature* 256: 495-497 (1975); Kozbor *et al.*, *Immunology Today* 4: 72 (1983); Cole *et al.*, pg. 77-96 in *MONOCLONAL ANTIBODIES AND CANCER THERAPY*, Alan R. Liss, Inc. (1985).

25 Techniques for the production of single chain antibodies (U.S. Patent No. 4,946,778) can be adapted to produce single chain antibodies to polypeptides of this invention. Also, transgenic mice, or other organisms such as other mammals, may be used to express humanized antibodies.

30 Alternatively phage display technology may be utilized to select antibody genes with binding activities towards the polypeptide either from repertoires of PCR amplified v-genes of lymphocytes from humans screened for possessing anti-secA2 or from naive libraries (McCafferty, J. *et al.*, (1990), *Nature* 348, 552-554; Marks, J. *et al.*, (1992) *Biotechnology* 10, 779-783). The affinity of these antibodies can also be improved by chain shuffling (Clackson, T. *et al.*, (1991) *Nature* 352, 624-628).

35 If two antigen binding domains are present each domain may be directed against a different epitope - termed 'bispecific' antibodies.

The above-described antibodies may be employed to isolate or to identify clones expressing the polypeptides to purify the polypeptides by affinity chromatography.

40 Thus, among others, antibodies against secA2- polypeptide may be employed to treat infections, particularly bacterial infections and especially disease, such as, infections of the upper respiratory tract (e.g., otitis media, bacterial tracheitis, acute epiglottitis, thyroiditis), lower respiratory (e.g., empyema, lung abscess), cardiac (e.g., infective endocarditis), gastrointestinal (e.g., secretory diarrhoea, splenic abscess, retroperitoneal abscess), CNS (e.g., cerebral abscess), eye (e.g., blepharitis, conjunctivitis, keratitis, endophthalmitis, preseptal and orbital cellulitis, dacryocystitis), kidney and urinary tract (e.g., epididymitis, intrarenal and perinephric abscess, toxic shock syndrome), skin (e.g., impetigo, folliculitis, cutaneous abscesses, cellulitis, wound infection, bacterial myositis) bone and joint (e.g., septic arthritis, osteomyelitis).

45 Polypeptide variants include antigenically, epitopically or immunologically equivalent variants that form a particular aspect of this invention. The term "antigenically equivalent derivative" as used herein encompasses a polypeptide or its equivalent which will be specifically recognized by certain antibodies which, when raised to the protein or polypeptide according to the invention, interfere with the immediate physical interaction between pathogen and mammalian host. The term "immunologically equivalent derivative" as used herein encompasses a peptide or its equivalent which when used in a suitable formulation to raise antibodies in a vertebrate, the antibodies act to interfere with the immediate physical interaction between pathogen and mammalian host.

50 The polypeptide, such as an antigenically or immunologically equivalent derivative or a fusion protein thereof is used as an antigen to immunize a mouse or other animal such as a rat or chicken. The fusion protein may provide stability to the polypeptide. The antigen may be associated, for example by conjugation, with an immunogenic carrier protein for example bovine serum albumin (BSA) or keyhole limpet haemocyanin (KLH). Alternatively a multiple antigenic peptide comprising multiple copies of the protein or polypeptide, or an antigenically or immunologically equivalent

polypeptide thereof may be sufficiently antigenic to improve immunogenicity so as to obviate the use of a carrier.

Preferably, the antibody or variant thereof is modified to make it less immunogenic in the individual. For example, if the individual is human the antibody may most preferably be "humanized"; where the complementarity determining region(s) of the hybridoma-derived antibody has been transplanted into a human monoclonal antibody, for example as described in Jones, P. et al. (1986), *Nature* 321, 522-525 or Tempest et al., (1991) *Biotechnology* 9, 266-273.

The use of a polynucleotide of the invention in genetic immunization will preferably employ a suitable delivery method such as direct injection of plasmid DNA into muscles (Wolff et al., *Hum Mol Genet* 1992, 1:363, Manthorpe et al., *Hum. Gene Ther.* 1993:4, 419), delivery of DNA complexed with specific protein carriers (Wu et al., *J Biol Chem.* 1989: 264,16985), coprecipitation of DNA with calcium phosphate (Benvenisty & Reshef, *PNAS USA*, 1986:83,9551), encapsulation of DNA in various forms of liposomes (Kaneda et al., *Science* 1989:243,375), particle bombardment (Tang et al., *Nature* 1992, 356:152, Eisenbraun et al., *DNA Cell Biol* 1993, 12:791) and *in vivo* infection using cloned retroviral vectors (Seeger et al., *PNAS USA* 1984:81,5849).

Antagonists and agonists - assays and molecules

Polypeptides of the invention may also be used to assess the binding of small molecule substrates and ligands in, for example, cells, cell-free preparations, chemical libraries, and natural product mixtures. These substrates and ligands may be natural substrates and ligands or may be structural or functional mimetics. See, e.g., Coligan et al., *Current Protocols in Immunology* 1(2): Chapter 5 (1991).

The invention also provides a method of screening compounds to identify those which enhance (agonist) or block (antagonist) the action of secA2 polypeptides or polynucleotides, particularly those compounds that are bacteriostatic and/or bacteriocidal. The method of screening may involve high-throughput techniques. For example, to screen for agonists or antagonists, a synthetic reaction mix, a cellular compartment, such as a membrane, cell envelope or cell wall, or a preparation of any thereof, comprising secA2 polypeptide and a labeled substrate or ligand of such polypeptide is incubated in the absence or the presence of a candidate molecule that may be a secA2 agonist or antagonist. The ability of the candidate molecule to agonize or antagonize the secA2 polypeptide is reflected in decreased binding of the labeled ligand or decreased production of product from such substrate. Molecules that bind gratuitously, i.e., without inducing the effects of secA2 polypeptide are most likely to be good antagonists. Molecules that bind well and increase the rate of product production from substrate are agonists. Detection of the rate or level of production of product from substrate may be enhanced by using a reporter system. Reporter systems that may be useful in this regard include but are not limited to colorimetric labeled substrate converted into product, a reporter gene that is responsive to changes in secA2 polynucleotide or polypeptide activity, and binding assays known in the art.

Another example of an assay for secA2 antagonists is a competitive assay that combines secA2 and a potential antagonist with secA2-binding molecules, recombinant secA2 binding molecules, natural substrates or ligands, or substrate or ligand mimetics, under appropriate conditions for a competitive inhibition assay. secA2 can be labeled, such as by radioactivity or a colorimetric compound, such that the number of secA2 molecules bound to a binding molecule or converted to product can be determined accurately to assess the effectiveness of the potential antagonist.

Potential antagonists include small organic molecules, peptides, polypeptides and antibodies that bind to a polynucleotide or polypeptide of the invention and thereby inhibit or extinguish its activity. Potential antagonists also may be small organic molecules, a peptide, a polypeptide such as a closely related protein or antibody that binds the same sites on a binding molecule, such as a binding molecule, without inducing secA2-induced activities, thereby preventing the action of secA2 by excluding secA2 from binding.

Potential antagonists include a small molecule that binds to and occupies the binding site of the polypeptide thereby preventing binding to cellular binding molecules, such that normal biological activity is prevented. Examples of small molecules include but are not limited to small organic molecules, peptides or peptide-like molecules. Other potential antagonists include antisense molecules (see Okano, *J. Neurochem.* 56: 560 (1991); *OLIGODEOXYNUCLEOTIDES AS ANTISENSE INHIBITORS OF GENE EXPRESSION*, CRC Press, Boca Raton, FL (1988), for a description of these molecules). Preferred potential antagonists include compounds related to and variants of secA2.

Each of the DNA sequences provided herein may be used in the discovery and development of antibacterial compounds. The encoded protein, upon expression, can be used as a target for the screening of antibacterial drugs. Additionally, the DNA sequences encoding the amino terminal regions of the encoded protein or Shine-Delgarno or other translation facilitating sequences of the respective mRNA can be used to construct antisense sequences to control the expression of the coding sequence of interest.

The invention also provides the use of the polypeptide, polynucleotide or inhibitor of the invention to interfere with the initial physical interaction between a pathogen and mammalian host responsible for sequelae of infection. In particular the molecules of the invention may be used: in the prevention of adhesion of bacteria, in particular gram positive bacteria, to mammalian extracellular matrix proteins on in-dwelling devices or to extracellular matrix proteins in wounds; to block secA2 protein-mediated mammalian cell invasion by, for example, initiating phosphorylation of mammalian

tyrosine kinases (Rosenshine *et al.*, *Infect. Immun.* 60:2211 (1992); to block bacterial adhesion between mammalian extracellular matrix proteins and bacterial secA2 proteins that mediate tissue damage and; to block the normal progression of pathogenesis in infections initiated other than by the implantation of in-dwelling devices or by other surgical techniques.

The antagonists and agonists of the invention may be employed, for instance, to inhibit and treat disease, such as, infections of the upper respiratory tract (e.g., otitis media, bacterial tracheitis, acute epiglottitis, thyroiditis), lower respiratory (e.g., empyema, lung abscess), cardiac (e.g., infective endocarditis), gastrointestinal (e.g., secretory diarrhoea, splenic abscess, retroperitoneal abscess), CNS (e.g., cerebral abscess), eye (e.g., blepharitis, conjunctivitis, keratitis, endophthalmitis, preseptal and orbital cellulitis, dacryocystitis), kidney and urinary tract (e.g., epididymitis, intrarenal and perinephric abscess, toxic shock syndrome), skin (e.g., impetigo, folliculitis, cutaneous abscesses, cellulitis, wound infection, bacterial myositis) bone and joint (e.g., septic arthritis, osteomyelitis).

Vaccines

Another aspect of the invention relates to a method for inducing an immunological response in an individual, particularly a mammal which comprises inoculating the individual with secA2, or a fragment or variant thereof, adequate to produce antibody and/ or T cell immune response to protect said individual from infection, particularly bacterial infection and most particularly *Staphylococcus aureus* infection. Also provided are methods whereby such immunological response slows bacterial replication. Yet another aspect of the invention relates to a method of inducing immunological response in an individual which comprises delivering to such individual a nucleic acid vector to direct expression of secA2, or a fragment or a variant thereof, for expressing secA2, or a fragment or a variant thereof *in vivo* in order to induce an immunological response, such as, to produce antibody and/ or T cell immune response, including, for example, cytokine-producing T cells or cytotoxic T cells, to protect said individual from disease, whether that disease is already established within the individual or not. One way of administering the gene is by accelerating it into the desired cells as a coating on particles or otherwise.

Such nucleic acid vector may comprise DNA, RNA, a modified nucleic acid, or a DNA/RNA hybrid.

A further aspect of the invention relates to an immunological composition which, when introduced into an individual capable of having induced within it an immunological response, induces an immunological response in such individual to a secA2 or protein coded therefrom, wherein the composition comprises a recombinant secA2 or protein coded therefrom comprising DNA which codes for and expresses an antigen of said secA2 or protein coded therefrom. The immunological response may be used therapeutically or prophylactically and may take the form of antibody immunity or cellular immunity such as that arising from CTL or CD4+ T cells.

A secA2 polypeptide or a fragment thereof may be fused with co-protein which may not by itself produce antibodies, but is capable of stabilizing the first protein and producing a fused protein which will have immunogenic and protective properties. Thus fused recombinant protein, preferably further comprises an antigenic co-protein, such as lipoprotein D from *Haemophilus influenzae*, Glutathione-S-transferase (GST) or beta-galactosidase, relatively large co-proteins which solubilize the protein and facilitate production and purification thereof. Moreover, the co-protein may act as an adjuvant in the sense of providing a generalized stimulation of the immune system. The co-protein may be attached to either the amino or carboxy terminus of the first protein.

Provided by this invention are compositions, particularly vaccine compositions, and methods comprising the polypeptides or polynucleotides of the invention and immunostimulatory DNA sequences, such as those described in Sato, Y. *et al.* Science 273: 352 (1996).

Also, provided by this invention are methods using the described polynucleotide or particular fragments thereof which have been shown to encode non-variable regions of bacterial cell surface proteins in DNA constructs used in such genetic immunization experiments in animal models of infection with *Staphylococcus aureus* will be particularly useful for identifying protein epitopes able to provoke a prophylactic or therapeutic immune response. It is believed that this approach will allow for the subsequent preparation of monoclonal antibodies of particular value from the requisite organ of the animal successfully resisting or clearing infection for the development of prophylactic agents or therapeutic treatments of bacterial infection, particularly *Staphylococcus aureus* infection, in mammals, particularly humans.

The polypeptide may be used as an antigen for vaccination of a host to produce specific antibodies which protect against invasion of bacteria, for example by blocking adherence of bacteria to damaged tissue. Examples of tissue damage include wounds in skin or connective tissue caused, e.g., by mechanical, chemical or thermal damage or by implantation of indwelling devices, or wounds in the mucous membranes, such as the mouth, mammary glands, urethra or vagina.

The invention also includes a vaccine formulation which comprises an immunogenic recombinant protein of the invention together with a suitable carrier. Since the protein may be broken down in the stomach, it is preferably administered parenterally, including, for example, administration that is subcutaneous, intramuscular, intravenous, or intra-

dermal. Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the bodily fluid, preferably the blood, of the individual; and aqueous and non-aqueous sterile suspensions which may include suspending agents or thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampules and vials and may be stored in a freeze-dried condition requiring only the addition of the sterile liquid carrier immediately prior to use. The vaccine formulation may also include adjuvant systems for enhancing the immunogenicity of the formulation, such as oil-in water systems and other systems known in the art. The dosage will depend on the specific activity of the vaccine and can be readily determined by routine experimentation.

While the invention has been described with reference to certain secA2 protein, it is to be understood that this covers fragments of the naturally occurring protein and similar proteins with additions, deletions or substitutions which do not substantially affect the immunogenic properties of the recombinant protein.

Compositions, kits and administration

The invention also relates to compositions comprising the polynucleotide or the polypeptides discussed above or their agonists or antagonists. The polypeptides of the invention may be employed in combination with a non-sterile or sterile carrier or carriers for use with cells, tissues or organisms, such as a pharmaceutical carrier suitable for administration to a subject. Such compositions comprise, for instance, a media additive or a therapeutically effective amount of a polypeptide of the invention and a pharmaceutically acceptable carrier or excipient. Such carriers may include, but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol and combinations thereof. The formulation should suit the mode of administration. The invention further relates to diagnostic and pharmaceutical packs and kits comprising one or more containers filled with one or more of the ingredients of the aforementioned compositions of the invention.

Polypeptides and other compounds of the invention may be employed alone or in conjunction with other compounds, such as therapeutic compounds.

The pharmaceutical compositions may be administered in any effective, convenient manner including, for instance, administration by topical, oral, anal, vaginal, intravenous, intraperitoneal, intramuscular, subcutaneous, intranasal or intradermal routes among others.

In therapy or as a prophylactic, the active agent may be administered to an individual as an injectable composition, for example as a sterile aqueous dispersion, preferably isotonic.

Alternatively the composition may be formulated for topical application for example in the form of ointments, creams, lotions, eye ointments, eye drops, ear drops, mouthwash, impregnated dressings and sutures and aerosols, and may contain appropriate conventional additives, including, for example, preservatives, solvents to assist drug penetration, and emollients in ointments and creams. Such topical formulations may also contain compatible conventional carriers, for example cream or ointment bases, and ethanol or oleyl alcohol for lotions. Such carriers may constitute from about 1% to about 98% by weight of the formulation; more usually they will constitute up to about 80% by weight of the formulation.

For administration to mammals, and particularly humans, it is expected that the daily dosage level of the active agent will be from 0.01 mg/kg to 10 mg/kg, typically around 1 mg/kg. The physician in any event will determine the actual dosage which will be most suitable for an individual and will vary with the age, weight and response of the particular individual. The above dosages are exemplary of the average case. There can, of course, be individual instances where higher or lower dosage ranges are merited, and such are within the scope of this invention.

In-dwelling devices include surgical implants, prosthetic devices and catheters, i.e., devices that are introduced to the body of an individual and remain in position for an extended time. Such devices include, for example, artificial joints, heart valves, pacemakers, vascular grafts, vascular catheters, cerebrospinal fluid shunts, urinary catheters, continuous ambulatory peritoneal dialysis (CAPD) catheters.

The composition of the invention may be administered by injection to achieve a systemic effect against relevant bacteria shortly before insertion of an in-dwelling device. Treatment may be continued after surgery during the in-body time of the device. In addition, the composition could also be used to broaden perioperative cover for any surgical technique to prevent bacterial wound infections, especially *Staphylococcus aureus* wound infections.

Many orthopaedic surgeons consider that humans with prosthetic joints should be considered for antibiotic prophylaxis before dental treatment that could produce a bacteremia. Late deep infection is a serious complication sometimes leading to loss of the prosthetic joint and is accompanied by significant morbidity and mortality. It may therefore be possible to extend the use of the active agent as a replacement for prophylactic antibiotics in this situation.

In addition to the therapy described above, the compositions of this invention may be used generally as a wound treatment agent to prevent adhesion of bacteria to matrix proteins exposed in wound tissue and for prophylactic use in dental treatment as an alternative to, or in conjunction with, antibiotic prophylaxis.

Alternatively, the composition of the invention may be used to bathe an indwelling device immediately before in-

section. The active agent will preferably be present at a concentration of 1µg/ml to 10mg/ml for bathing of wounds or indwelling devices.

A vaccine composition is conveniently in injectable form. Conventional adjuvants may be employed to enhance the immune response. A suitable unit dose for vaccination is 0.5-5 microgram/kg of antigen, and such dose is preferably administered 1-3 times and with an interval of 1-3 weeks. With the indicated dose range, no adverse toxicological effects will be observed with the compounds of the invention which would preclude their administration to suitable individuals.

Each reference disclosed herein is incorporated by reference herein in its entirety. Any patent application to which this application claims priority is also incorporated by reference herein in its entirety.

EXAMPLES

The examples below are carried out using standard techniques, which are well known and routine to those of skill in the art, except where otherwise described in detail. The examples are illustrative, but do not limit the invention.

Example 1 Strain selection, Library Production and Sequencing

The polynucleotide having the DNA sequence given in SEQ ID NO:1 was obtained from a library of clones of chromosomal DNA of *Staphylococcus aureus* in *E. coli*. The sequencing data from two or more clones containing overlapping *Staphylococcus aureus* DNAs was used to construct the contiguous DNA sequence in SEQ ID NO:1. Libraries may be prepared by routine methods, for example:

Methods 1 and 2 below.

Total cellular DNA is isolated from *Staphylococcus aureus* WCUH 29 according to standard procedures and size-fractionated by either of two methods.

Method 1

Total cellular DNA is mechanically sheared by passage through a needle in order to size-fractionate according to standard procedures. DNA fragments of up to 11kbp in size are rendered blunt by treatment with exonuclease and DNA polymerase, and EcoRI linkers added. Fragments are ligated into the vector Lambda ZapII that has been cut with EcoRI, the library packaged by standard procedures and *E. coli* infected with the packaged library. The library is amplified by standard procedures.

Method 2

Total cellular DNA is partially hydrolyzed with a one or a combination of restriction enzymes appropriate to generate a series of fragments for cloning into library vectors (e.g., RsaI, PstI, AluI, Bsh1235I), and such fragments are size-fractionated according to standard procedures. EcoRI linkers are ligated to the DNA and the fragments then ligated into the vector Lambda ZapII that have been cut with EcoRI, the library packaged by standard procedures, and *E. coli* infected with the packaged library. The library is amplified by standard procedures.

Example 2 secA2 Characterization

This assay involves a modification of the *E. coli* *in vitro* translocation assay which is well documented in the literature (11,12,13). Inside-out membrane vesicles from *S. aureus* are prepared as described by Schimz *et al.* (14). Purified SecA2 is added to urea washed membrane vesicles in the presence of Mg-ATP and test compound and the reaction started by the addition of a small volume of an appropriate preprotein substrate (for example, proOmpA (11,12,13) or preprolipase(14)). The inorganic phosphate released by ATP hydrolysis is then quantified by a colorimetric assay described by Lanzetta *et al.* (15). Thus inhibitors of SecA2 can be detected by a reduction in amount of inorganic phosphate produced relative to the control.

References:

13. Cabelli, R.J. *et al.* (1988) *Cell* 55, 683-692.
14. Cunningham, K. *et al.* (1989) *The EMBO Journal* 8, 955-959.
15. Lill, R. *et al.* (1990) *Cell* 60, 271-280.
16. Schimz, K.-L. *et al.* (1995) *FEBS Letters* 362, 29-33.

17. Lanzetta, P.A, et al. (1979) Analytical Biochemistry 100, 95-97.

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Ann x t th description

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: SmithKline Beecham Corporation

(B) STREET: Two New Horizons Court

(C) CITY: Brentford

(D) STATE OR PROVINCE: Middlesex

(E) COUNTRY: United Kingdom

(F) POSTAL CODE: TW8 9EP

(ii) TITLE OF INVENTION: Novel Compounds

(iii) NUMBER OF SEQUENCES: 6

(iv) COMPUTER-READABLE FORM:

(A) MEDIUM TYPE: Diskette

(B) COMPUTER: IBM Compatible

(C) OPERATING SYSTEM: Windows 95

(D) SOFTWARE: FastSEQ for Windows Version 2.0b

(v) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2388 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

5 ATGAAACATA AACTAGATGT TACGATAAAC GAATTAAGAC TAAAGTCGAT
TAGAAAAATA 60
10 GTGAAACGCA TCAATACATG GAGCGATGAA GTTAAATCAT ATTCTGATGA
TGC GTTAAAA 120
15 CAAAAGACAA TAGAATTTAA AGAACGTTTA GCATCAGGAG TTGATACACT
AGATACATTG 180
20 TTACCTGAAG CTTATGCAGT GGCACGTGAA GCGAGCTGGA GAGTATTAGG
TATGTATCCT 240
25 AAAGAAGTTC AGTTAATCGG AGCGATTGTA TTGCATGAAG GTAACATTGC
GGAGATGCAA 300
30 ACAGGCGAAG GTAAAACATT AACGGCAACG ATGCCTTTAT ATTTAAATGC
TCTTTCGGGA 360
35 AAAGGAACAT ATTTAATTAC AACGAATGAT TATTTAGCGA AGCGAGATTT
TGAAGAGATG 420
40 CAACCATTAT ATGAATGGTT AGGTTTAACT GCTTCATTAG GTTTTGTTGA
TATTGTGGAT 480
45 TATGAGTATC AAAAAGGGGA AAAACGTAAT ATATATGAAC ATGATATTAT
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GGAAGGGAAA 600
55 TTTTACCAC AATTAAATTA CGGTATTATT GATGAAGTGG ATTCAATCAT
TTTAGATGCT 660
60 GCTCAAACAC CATTAGTTAT TTCGGGTGCA CCAAGATTAC AATCAAATCT
ATTCATATT 720
65 GTGAAAGAGT TTGTAGATAC ATTGATTGAA GACGTGCATT TAAAATGAA
GAAGACCAA 780
70 AAAGAAATAT GGCTGTAAA TCAAGGTATT GAAGCGGCAC AATCATACTT
TAATGTTGAA 840

5 GATTTATATA GCGAACAAGC GATGGTCCTA GTGCGTAATA TTAATTTAGC
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 CAATATTTGT TTGAATCTAA TGTCGATTAC TTTGTATATA ATGGTGATAT
 TGTTTTAATT 960
 10 GACCGTATTA CAGGTCGTAT GTTACCGGGA ACTAAGTTGC AAGCTGGACT
 TCACCAAGCT 1020
 ATTGAAGCGA AAGAAGGTAT GGAGGTTTCA ACAGATAAAA GTGTTATGGC
 15 AACAATTACC 1080
 TTCCAGAATT TATTTAAACT TTTTGAATCA TTTTCAGGTA TGACAGCTAC
 AGGAAAATTA 1140
 20 GGCGAATCAG AGTTCTTTGA TTTGTATTCA AAAATAGTCG TACAAGCACC
 AACTGATAAA 1200
 GCGATTCAAC GTATCGATGA ACCAGATAAA GTGTTTCGTT CAGTTGATGA
 25 GAAAAACATC 1260
 GCGATGATTC ATGATATAGT TGAAC TTCAT GAAACGGGGC GACCGGTTTT
 ACTCATAACG 1320
 30 AGAACTGCTG AAGCGGCTGA ATACTTTTCC GAAGTATTAT TCCAAATGGA
 TATTCCTAAT 1380
 AATTTACTCA TTGCGCAAAA TGTGCAAAA GAAGCGCAGA TGATAGCTGA
 AGCAGGGCAA 1440
 35 ATTGGTTCCA TGACTGTTGC GACTAGTATG GCAGGTCGAG GCACAGATAT
 TAAACTTGGT 1500
 GAAGGTGTCG AAGCATTAGG TGGATTAGCT GTTATTATTC ATGAACATAT
 40 GGAAAATAGC 1560
 CGTGTAGACA GGCAATTACG TGGTCGTTCT GGTAGACAAG GGGATCCGGG
 ATCATCTTGT 1620
 45 ATATATATTT CACTAGATGA TTATTTAGTT AAGCGATGGA GCGATAGTAA
 TTTAGCGGAA 1680
 AATAATCAAT TATATTCATT AGATGCACAA CGATTATCGC AAAGTAATTI
 50 GTTTAATCGC 1740
 AAAGTTAAGC AAATTGTAGT TAAAGCGCAG CGTATCTCGG AAGAACAAGG
 GGTTAAAGCT 1800

CGTGAAATGG CTAATGAATT TGAAAAAAGC ATTAGTATAC AGCGAGATCT
TGTATACGAG 1860

GAACGCAATC GAGTTTTAGA AATAGATGAT GCTGAGAATC GAGATTTTAA
AGCGTTAGCT 1920

AAAGATGTAT TTGAAATGTT TGTAATGAG GAAAAGGTGC TAACAAAATC
GCGTGTTGTC 1980

GAGTATATTT ATCAAAATTT AAGTTTCCAA TTTAATAAAG ATGTGGCTTG
TGTTAATTTT 2040

AAAGATAAGC AAGCAGTAGT GACATTTTAA TTAGAGCAAT TTGAAAAGCA
ATTAGCTTTG 2100

AATCGTAAAA ACATGCAAAG TGCATATTAT TATAATATTT TCGTTCAAAA
AGTCTTTTTG 2160

AAAGCAATTG ATTCATGTTG GTTAGAACAA GTCGACTATT TACAACAATT
AAAAGCAAGC 2220

GTTAATCAAC GACAAAATGG GCAACGTAAC GCTATTTTTG AATACCATAG
AGTTGCATTA 2280

GATTCATTG AAGTTATGAC ACGAAATATC AAAAAAAGAA TGGTGAAAAA
TATTTGTCAA 2340

AGCATGATTA CATTTGATAA AGAGGGTATG CCAGTTATAC ATTTTCCA
2388

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 796 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Lys His Lys Leu Asp Val Thr Ile Asn Glu Leu Arg Leu Lys Ser

1 5 10 15
 5 Ile Arg Lys Ile Val Lys Arg Ile Asn Thr Trp Ser Asp Glu Val Lys
 20 25 30
 Ser Tyr Ser Asp Asp Ala Leu Lys Gln Lys Thr Ile Glu Phe Lys Glu
 10 35 40 45
 Arg Leu Ala Ser Gly Val Asp Thr Leu Asp Thr Leu Leu Pro Glu Ala
 50 55 60
 15 Tyr Ala Val Ala Arg Glu Ala Ser Trp Arg Val Leu Gly Met Tyr Pro
 65 70 75 80
 Lys Glu Val Gln Leu Ile Gly Ala Ile Val Leu His Glu Gly Asn Ile
 85 90 95
 20 Ala Glu Met Gln Thr Gly Glu Gly Lys Thr Leu Thr Ala Thr Met Pro
 100 105 110
 Leu Tyr Leu Asn Ala Leu Ser Gly Lys Gly Thr Tyr Leu Ile Thr Thr
 25 115 120 125
 Asn Asp Tyr Leu Ala Lys Arg Asp Phe Glu Glu Met Gln Pro Leu Tyr
 130 135 140
 30 Glu Trp Leu Gly Leu Thr Ala Ser Leu Gly Phe Val Asp Ile Val Asp
 145 150 155 160
 Tyr Glu Tyr Gln Lys Gly Glu Lys Arg Asn Ile Tyr Glu His Asp Ile
 35 165 170 175
 Ile Tyr Thr Thr Asn Gly Arg Leu Gly Phe Asp Tyr Leu Ile Asp Asn
 180 185 190
 40 Leu Ala Asp Ser Ala Glu Gly Lys Phe Leu Pro Gln Leu Asn Tyr Gly
 195 200 205
 Ile Ile Asp Glu Val Asp Ser Ile Ile Leu Asp Ala Ala Gln Thr Pro
 45 210 215 220
 Leu Val Ile Ser Gly Ala Pro Arg Leu Gln Ser Asn Leu Phe His Ile
 225 230 235 240
 50 Val Lys Glu Phe Val Asp Thr Leu Ile Glu Asp Val His Phe Lys Met
 245 250 255
 Lys Lys Thr Lys Lys Glu Ile Trp Leu Leu Asn Gln Gly Ile Glu Ala
 55 260 265 270

EP 0 892 064 A2

Ala Gln Ser Tyr Phe Asn Val Glu Asp Leu Tyr Ser Glu Gln Ala Met
275 280 285
5 Val Leu Val Arg Asn Ile Asn Leu Ala Leu Arg Ala Gln Tyr Leu Phe
290 295 300
10 Glu Ser Asn Val Asp Tyr Phe Val Tyr Asn Gly Asp Ile Val Leu Ile
305 310 315 320
Asp Arg Ile Thr Gly Arg Met Leu Pro Gly Thr Lys Leu Gln Ala Gly
325 330 335
15 Leu His Gln Ala Ile Glu Ala Lys Glu Gly Met Glu Val Ser Thr Asp
340 345 350
Lys Ser Val Met Ala Thr Ile Thr Phe Gln Asn Leu Phe Lys Leu Phe
20 355 360 365
Glu Ser Phe Ser Gly Met Thr Ala Thr Gly Lys Leu Gly Glu Ser Glu
370 375 380
25 Phe Phe Asp Leu Tyr Ser Lys Ile Val Val Gln Ala Pro Thr Asp Lys
385 390 395 400
Ala Ile Gln Arg Ile Asp Glu Pro Asp Lys Val Phe Arg Ser Val Asp
30 405 410 415
Glu Lys Asn Ile Ala Met Ile His Asp Ile Val Glu Leu His Glu Thr
420 425 430
35 Gly Arg Pro Val Leu Leu Ile Thr Arg Thr Ala Glu Ala Ala Glu Tyr
435 440 445
Phe Ser Glu Val Leu Phe Gln Met Asp Ile Pro Asn Asn Leu Leu Ile
450 455 460
40 Ala Gln Asn Val Ala Lys Glu Ala Gln Met Ile Ala Glu Ala Gly Gln
465 470 475 480
Ile Gly Ser Met Thr Val Ala Thr Ser Met Ala Gly Arg Gly Thr Asp
45 485 490 495
Ile Lys Leu Gly Glu Gly Val Glu Ala Leu Gly Gly Leu Ala Val Ile
500 505 510
50 Ile His Glu His Met Glu Asn Ser Arg Val Asp Arg Gln Leu Arg Gly
515 520 525
Arg Ser Gly Arg Gln Gly Asp Pro Gly Ser Ser Cys Ile Tyr Ile Ser
55

530 535 540
 5 Leu Asp Asp Tyr Leu Val Lys Arg Trp Ser Asp Ser Asn Leu Ala Glu
 545 550 555 560
 Asn Asn Gln Leu Tyr Ser Leu Asp Ala Gln Arg Leu Ser Gln Ser Asn
 10 565 570 575
 Leu Phe Asn Arg Lys Val Lys Gln Ile Val Val Lys Ala Gln Arg Ile
 580 585 590
 15 Ser Glu Glu Gln Gly Val Lys Ala Arg Glu Met Ala Asn Glu Phe Glu
 595 600 605
 Lys Ser Ile Ser Ile Gln Arg Asp Leu Val Tyr Glu Glu Arg Asn Arg
 610 615 620
 20 Val Leu Glu Ile Asp Asp Ala Glu Asn Arg Asp Phe Lys Ala Leu Ala
 625 630 635 640
 Lys Asp Val Phe Glu Met Phe Val Asn Glu Glu Lys Val Leu Thr Lys
 25 645 650 655
 Ser Arg Val Val Glu Tyr Ile Tyr Gln Asn Leu Ser Phe Gln Phe Asn
 660 665 670
 30 Lys Asp Val Ala Cys Val Asn Phe Lys Asp Lys Gln Ala Val Val Thr
 675 680 685
 Phe Leu Leu Glu Gln Phe Glu Lys Gln Leu Ala Leu Asn Arg Lys Asn
 35 690 695 700
 Met Gln Ser Ala Tyr Tyr Tyr Asn Ile Phe Val Gln Lys Val Phe Leu
 705 710 715 720
 40 Lys Ala Ile Asp Ser Cys Trp Leu Glu Gln Val Asp Tyr Leu Gln Gln
 725 730 735
 Leu Lys Ala Ser Val Asn Gln Arg Gln Asn Gly Gln Arg Asn Ala Ile
 45 740 745 750
 Phe Glu Tyr His Arg Val Ala Leu Asp Ser Phe Glu Val Met Thr Arg
 755 760 765
 50 Asn Ile Lys Lys Arg Met Val Lys Asn Ile Cys Gln Ser Met Ile Thr
 770 775 780
 Phe Asp Lys Glu Gly Met Pro Val Ile His Phe Pro
 55 785 790 795

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1496 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GTTAATCAAG TATCGAAGCG GAACAATCAT ACTTTAATGT TGAAGATTTA
TATNGCGAAC 60
AAGCGATGGT CCTAGTGCGT AATATTAATT TAGCACTGCG CGCACAATAT
TTGTTNGNAT 120
CTNATGTCGA TTACTTTGTA TATNNTGGTG ATATTGTTTT AACTGACCNC
ATTACAGGTC 180
GTNTGTTACC GGNAACTAAG TTGCAAGCTG GACTTCACCA NGCTATTGAA
GCGAAAGAAG 240
GTATGGAGGT TTCAACAGAT AAAAGTGTTA TGCCAACCAA TTACCCTTCC
AGAATTTATT 300
TAAACTTTTT GAATCAATTT TCAGGTATGA CAAGCTACAG GAAAATTAGG
CGAATCAGAG 360
TTCTTTGATT TGTATTCANA AATAGTCGTA CAAGCACCCA ACTGATAAAG
CGATTCAACG 420
TATCGATGAA CCAGATAAAG TGTTCGTTC AGTTGATGAG AAAAACATCG
CGATGATTCA 480
TTGATATAGT TGAAGTTCAT GANNCGGGGC CGACCGGTTT TACCTCATAA
CCGAGNACTG 540
CTGAAGCGGC TTGAATACTT TTCNGAAGTA TTATTCCAAA TGGATATTCC
TAATAATTTA 600

CTCATTGCGC AAAATGTTCC AAAAGAAGCG CAGATGATAG CTGAAGCAGG
CCAAATTGGT 660

TCCATGACTG TTGCGACTAG TATGGCAGGT CGAGGCACAG ATATTAAACT
TGGTGAAGGT 720

GTCGAAGCAT TAGCTGGATT AGCTGTTATT ATTCATGAAC ATATGGAAAA
TAGCCGTGTA 780

GACAGGCAAT TACGTGGTCG TTCTGGTAGA CAAGGGGATC CGGGATCATC
TTGTATATAT 840

ATTTCACTAG ATGATTATTT AGNTAAGCGA TGGAGCGATA GTAATTTAGC
GGAAAATAAT 900

CAATTATATT CANTAGATGC ACAACGATTA TCGCAAAGTA ATTTGTTTAA
TCGNAAAGTT 960

AAGCAAATTG TAGTTAAAGC GCAGCGTATC TCGGAAAGAA CAAGGGGTTA
AAGCTCGGTG 1020

AAATGGCTTA ATTGAATTTG NNAAAAAGCA TNAGTATTCA GCGAAGATCT
TNGTATTTAC 1080

GANGGAACGC AAATCCGAGT TTTTAGAAAT TAGATTGATG CTGAGAATCC
NAGATTTTTA 1140

ANGCGGTTAG CTAAAGATT GTATTTGAAA TNGTTTGGGG NAATGANGGA
AANGGTGCTA 1200

ACAAAATCGC GNGTTGGGCG AGTATATTTT ATCAAAAATT TAAGTTNCCA
ATTTAATAAA 1260

GATGTGGCTT GTGTTAATTT TAAAGATAAG CAAGCAGNAG TGACATTTTT
ATTAGAGCAA 1320

TTTGAAAAGC AATTAGCTTT GGANTCCGTA AAAACATGCA ANGNGCATAT
TATTATAATA 1380

TTNCCGGCCA AAANGTCTTT NGGGAAAGCA ATTGATNCAA GTTGGGGTTA
GGAACAAGTC 1440

GGCTTTTNAC AACAANTTAA NAGCAAGCGN TAATCAAACG ACAAANTGG
CAACCT 1496

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 143 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Asp Ile Pro Asn Asn Leu Leu Ile Ala Gln Asn Val Pro Lys Glu

1 5 10 15

Ala Gln Met Ile Ala Glu Ala Gly Gln Ile Gly Ser Met Thr Val Ala

20 25 30

Thr Ser Met Ala Gly Arg Gly Thr Asp Ile Lys Leu Gly Glu Gly Val

35 40 45

Glu Ala Leu Ala Gly Leu Ala Val Ile Ile His Glu His Met Glu Asn

50 55 60

Ser Arg Val Asp Arg Gln Leu Arg Gly Arg Ser Gly Arg Gln Gly Asp

65 70 75 80

Pro Gly Ser Ser Cys Ile Tyr Ile Ser Leu Asp Asp Tyr Leu Xaa Lys

85 90 95

Arg Trp Ser Asp Ser Asn Leu Ala Glu Asn Asn Gln Leu Tyr Ser Xaa

100 105 110

Asp Ala Gln Arg Leu Ser Gln Ser Asn Leu Phe Asn Arg Lys Val Lys

115 120 125

Gln Ile Val Val Lys Ala Gln Arg Ile Ser Glu Arg Thr Arg Gly

130 135 140

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

ATGAAACATA AACTAGATGT TACG

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(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

TGGAAAATGT ATAAGTGGCA TACC

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Claims

1. An isolated polynucleotide comprising a polynucleotide sequence selected from the group consisting of:

- (a) a polynucleotide having at least a 70% identity to a polynucleotide encoding a polypeptide comprising the amino acid sequence of SEQ ID NO:2;
- (b) a polynucleotide having at least a 70% identity to a polynucleotide encoding the same mature polypeptide expressed by the secA2 gene contained in the *Staphylococcus aureus* of the deposited strain;
- (c) a polynucleotide encoding a polypeptide comprising an amino acid sequence which is at least 70% identical to the amino acid sequence of SEQ ID NO:2;
- (d) a polynucleotide which is complementary to the polynucleotide of (a), (b) or (c); and
- (e) a polynucleotide comprising at least 15 sequential bases of the polynucleotide of (a), (b), (c) or (d).

2. The polynucleotide of Claim 1 wherein the polynucleotide is DNA.

3. The polynucleotide of Claim 1 wherein the polynucleotide is RNA.

4. The polynucleotide of Claim 2 comprising the nucleic acid sequence set forth in SEQ ID NO:1.

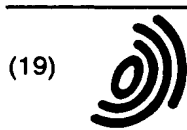
5. The polynucleotide of Claim 2 comprising nucleotide 1 to 2388 set forth in SEQ ID NO:1.

6. The polynucleotide of Claim 2 which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:2.
7. A vector comprising the polynucleotide of Claim 1.
8. A host cell comprising the vector of Claim 7.
9. A process for producing a polypeptide comprising: expressing from the host cell of Claim 8 a polypeptide encoded by said DNA.
10. A process for producing a secA2 polypeptide or fragment comprising culturing a host of claim 8 under conditions sufficient for the production of said polypeptide or fragment.
11. A polypeptide comprising an amino acid sequence which is at least 70% identical to the amino acid sequence of SEQ ID NO:2.
12. A polypeptide comprising an amino acid sequence as set forth in SEQ ID NO:2.
13. An antibody against the polypeptide of claim 11.
14. An antagonist which inhibits the activity or expression of the polypeptide of claim 11.
15. A method for the treatment of an individual in need of secA2 polypeptide comprising: administering to the individual a therapeutically effective amount of the polypeptide of claim 11.
16. A method for the treatment of an individual having need to inhibit secA2 polypeptide comprising: administering to the individual a therapeutically effective amount of the antagonist of Claim 14.
17. A process for diagnosing a disease related to expression or activity of the polypeptide of claim 11 in an individual comprising:
 - (a) determining a nucleic acid sequence encoding said polypeptide, and/or
 - (b) analyzing for the presence or amount of said polypeptide in a sample derived from the individual.
18. A method for identifying compounds which interact with and inhibit or activate an activity of the polypeptide of claim 11 comprising:

contacting a composition comprising the polypeptide with the compound to be screened under conditions to permit interaction between the compound and the polypeptide to assess the interaction of a compound, such interaction being associated with a second component capable of providing a detectable signal in response to the interaction of the polypeptide with the compound;

and determining whether the compound interacts with and activates or inhibits an activity of the polypeptide by detecting the presence or absence of a signal generated from the interaction of the compound with the polypeptide.
19. A method for inducing an immunological response in a mammal which comprises inoculating the mammal with secA2 polypeptide of claim 11, or a fragment or variant thereof, adequate to produce antibody and/or T cell immune response to protect said animal from disease.
20. A method of inducing immunological response in a mammal which comprises delivering a nucleic acid vector to direct expression of secA2 polypeptide of claim 11, or fragment or a variant thereof, for expressing said secA2 polypeptide, or a fragment or a variant thereof *in vivo* in order to induce an immunological response to produce antibody and/ or T cell immune response to protect said animal from disease.

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(71) Applicants:
• **SMITHKLINE BEECHAM CORPORATION**
Philadelphia Pennsylvania 19103 (US)
• **SMITHKLINE BEECHAM PLC**
Brentford, Middlesex TW8 9EP (GB)

(72) Inventors:
• **Warren, Richard, Jr.**
King of Prussia, Pennsylvania 19406 (US)
• **O'Dwyer, Karen**
Brentford Middlesex TW8 9EP (GB)
• **Perry, Caroline**
Brentford Middlesex TW8 9EP (GB)

(74) Representative: **Mallalieu, Catherine Louise et al**
D. Young & Co.,
21 New Fetter Lane
London EC4A 1DA (GB)

(54) **Recombinant secA2 polypeptide from Staphylococcus aureus and uses thereof**

(57) The invention provides secA2 polypeptides and DNA (RNA) encoding secA2 polypeptides and methods

for producing such polypeptides by recombinant techniques. Also provided are methods for utilizing secA2 polypeptides to screen for antibacterial compounds.

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Application Number

which under Rule 45 of the European Patent Convention EP 98 30 4311
shall be considered, for the purposes of subsequent
proceedings, as the European search report

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
X	<p>M KLEIN ET AL: "Functional characterization of S. carnosus SecA protein in E. coli and B. subtilis secA mutant strains"</p> <p>FEMS MICROBIOLOGY LETTERS, vol. 131, 1 January 1995 (1995-01-01), pages 271-277, XP002089426</p> <p>ISSN: 0378-1097</p> <p>Note: 61.4% nt sequence identity with SEQ ID NO:1 in 322 bp overlap, 36.9% aa sequence identity overlap with SEQ ID NO:2 in 791 aa overlap.</p> <p>* abstract *</p> <p>* figures 1,2 *</p> <p style="text-align: center;">---</p> <p style="text-align: center;">-/--</p>	<p>1-3, 7-10,13, 19,20</p>	<p>C12N15/55 C12N9/14 C12N5/10 C07K16/00 G01N33/50</p>
			<p>TECHNICAL FIELDS SEARCHED (Int.Cl.6)</p> <p>C12N C07K G01N</p>
<p>INCOMPLETE SEARCH</p> <p>The Search Division considers that the present application, or one or more of its claims, does/do not comply with the EPC to such an extent that a meaningful search into the state of the art cannot be carried out, or can only be carried out partially, for these claims.</p> <p>Claims searched completely :</p> <p>Claims searched incompletely :</p> <p>Claims not searched :</p> <p>Reason for the limitation of the search:</p> <p>see sheet C</p>			
Place of search		Date of completion of the search	Examiner
THE HAGUE		18 November 1999	van de Kamp, M
<p>CATEGORY OF CITED DOCUMENTS</p> <p>X : particularly relevant if taken alone</p> <p>Y : particularly relevant if combined with another document of the same category</p> <p>A : technological background</p> <p>O : non-written disclosure</p> <p>P : intermediate document</p> <p>T : theory or principle underlying the invention</p> <p>E : earlier patent document, but published on, or after the filing date</p> <p>D : document cited in the application</p> <p>L : document cited for other reasons</p> <p>& : member of the same patent family, corresponding document</p>			

EPO FORM 1503 03 82 (P4/C07)



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INCOMPLETE SEARCH
SHEET C

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Remark (1): Although claims 15, 19 and 20 are directed to a method of treatment of the human/animal body (Article 52(4) EPC), the search has been carried out and based on the alleged effects of the compound.

Claim(s) not searched:
14, 16

Reason for the limitation of the search:

Remark (2): Claim 14 and the dependent claim 16 refer to an antagonist of the polypeptide without giving a true technical characterisation. Moreover, no specific compounds are defined in the application. In consequence, the scope of said claims is ambiguous and vague, and their subject-matter is not sufficiently disclosed and supported (Art. 83 and 84 EPC). No search can be carried out for such purely speculative claims whose wording is, in fact, a mere recitation of the results to be achieved.



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Application Number
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DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int. CL6)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
A	<p>DESNOTTES J -F: "New targets and strategies for the development of antibacterial agents" TRENDS IN BIOTECHNOLOGY, vol. 14, no. 4, 1 April 1996 (1996-04-01), page 134-140 XP004035799 ISSN: 0167-7799 * page 134 * * page 137, right-hand column * * figure 3 *</p>	17,18	
A	<p>SADAIE Y ET AL.: "Sequencing reveals similarity of the wild-type div+ gene of Bacillus subtilis to the Escherichia coli secA gene" GENE, vol. 98, 1991, pages 101-105, XP002121321 Note: 54.7% nt sequence identity with SEQ ID NO:1 in 1164 bp overlap, 38.0% aa sequence identity with SEQ ID NO:2 in 787 aa overlap. * abstract * * figures 2,3 *</p>	1-13	TECHNICAL FIELDS SEARCHED (Int. CL6)
D,A	<p>WICKNER W ET AL.: "Escherichia coli preprotein translocase" J. BIOL. CHEM., vol. 271, 22 November 1996 (1996-11-22), pages 29514-29516, XP002121141 * the whole document *</p> <p style="text-align: center;">---</p> <p style="text-align: center;">-/--</p>	1-13	



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DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
A	OLIVER DB ET AL.: "Azide-resistant mutants of Escherichia coli alter the SecA protein, an azide-sensitive component of the protein export machinery" PROC. NATL. ACAD. SCI. USA, vol. 87, November 1990 (1990-11), pages 8227-8231, XP002121139 * abstract *		
P,X	EP 0 786 519 A (HUMAN GENOME SCIENCES INC) 30 July 1997 (1997-07-30) Note: 94.6% nt sequence identity of SEQ ID NO:220 with SEQ ID NO:1 in 2388 bp overlap, 96.4% aa sequence identity with SEQ ID NO:2 in 645 aa overlap. * page 4-7 * * page 9-21 * * page 25-26 * * page 60, line 220 * * page 972 - page 980 * * claims 8-11,14,15,18-22,28,29 *	1-13,15,17-20	TECHNICAL FIELDS SEARCHED (Int.Cl.6)
P,X	WO 97 31114 A (SMITHKLINE BEECHAM PLC ;BURNHAM MARTIN KARL RUSSELL (US); HODGSON) 28 August 1997 (1997-08-28) Note: 96.2% nt sequence identity of SEQ ID NO:37 with SEQ ID NO:1 in 792 bp overlap, 65.4% aa sequence identity with SEQ ID NO:2 in 318 aa overlap. * page 1-26 * * page 43-44 * * page 79-80 * * claims 1-23 *	1-3,7-10,13,19,20	

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EPO FORM 1500 (02/02) (P04CT18)



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Application Number
EP 98 30 4311

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION. (Int.Cl.6)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
E	<p>EP 0 882 795 A (SMITHKLINE BEECHAM PLC ;SMITHKLINE BEECHAM CORP (US)) 9 December 1998 (1998-12-09) Note: 65.2% nt sequence identity of SEQ ID NO:1 with SEQ ID NO:1 in 299 bp overlap, 39.0% aa sequence identity with SEQ ID NO:2 in 784 aa overlap. * the whole document * * page 6-8 * * page 21-25 * * claims 1-20 *</p> <p>-----</p>	1-3, 7-10,13, 19,20	
			TECHNICAL FIELDS SEARCHED (Int.Cl.6)

**ANNEX TO THE EUROPEAN SEARCH REPORT
ON EUROPEAN PATENT APPLICATION N° .**

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The members are as contained in the European Patent Office EDP file on
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18-11-1999

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0786519 A	30-07-1997	CA 2194411 A	06-07-1997
		JP 9322781 A	16-12-1997
WO 9731114 A	28-08-1997	EP 0822987 A	11-02-1998
		JP 11506022 T	02-06-1999
EP 0882795 A	09-12-1998	US 5891678 A	06-04-1999
		CA 2233587 A	04-12-1998
		JP 11098991 A	13-04-1999

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